Valve and girdle morphology of the deep-water chiton *Xylochiton xylophagus* Gowlett-Holmes & Jones, 1992 (Polyplacophora: Xylochitonidae).

A.M. Jones* and K.L. Gowlett-Holmes#

* Dept of Biological Sciences, The University, Dundee, DD1 4HN, Scotland
\# South Australian Museum, North Terrace, Adelaide, S. Aust. 5000

ABSTRACT

The morphology of the valves and girdle of the newly described, wood-feeding deep-water chiton *Xylochiton xylophagus* is examined using light and scanning electron microscopy. The aesthete groups are unusual in character and the subsidiary caps in particular are unique in being erect. The nature of the girdle spiculation is described in detail, and sparse, hair-like and probably sensory structures were observed; the ventral surface of the girdle is devoid of spicules, a feature found only in one other group of chitons, the Abyssochitonidae.

INTRODUCTION

The deep-water chitons of the monogeneric families Abyssochitonidae Dell'Angelo & Palazzi, 1989 and Xylochitonidae Gowlett-Holmes & Jones, 1992 are particularly interesting groups as most species described to date (Sirenko, 1988; Gowlett-Holmes and Jones, 1992) live on or in logs of sunken wood: that this forms the principal food source is demonstrated by the gut being full of wood fragments. Whether or not these animals are able to digest the wood itself, or whether they are feeding on associated microflora remains undetermined. These animals are also usually found in depths below about 700m where sunlight must be considered absent and, therefore, the structure and abundance of aesthetes, widely considered to be photoreceptor organs, is of special interest.

Xylochiton (family Xylochitonidae) is a monotypic genus with distinctive character differences from *Ferreiraella* (family Abyssochitonidae) (see Gowlett-Holmes & Jones, 1992). This paper describes the unusual valve and aesthete cap structures together with the girdle structures of *Xylochiton xylophagus* Gowlett-Holmes & Jones, 1992.

MATERIALS AND METHODS

All material of *Xylochiton xylophagus* examined here was originally brought to our attention by Mr B. Marshall of the National Museum of New Zealand, Wellington (NMNZ), and is now held by the Royal Scottish Museum, Edinburgh (RSM-NMSZ 1991055, paratypes). This material was obtained from a large sunken log of *Coriaria arborea* (Tree Tutu), at a depth of 1075-1100m, off White Island (37°23.7'S, 177°39.5-36.6'E), east of North Island, New Zealand, by the FV

"Kalinovo", 23 Nov. 1981, Stn BS 924 (K01/019/81). The chitons had been fixed in formalin solution and stored in 70% alcohol.

Valves were carefully dissected out from individual animals and then prepared for examination in one of several ways. For light microscopy, valves were examined by both direct and transmitted light on a Zeiss Stemi SV8 binocular microscope. Decalcified valves were prepared by treating with 5% disodium ethylenediaminetetra acetate (EDTA) solution until the calcareous material was solubilised. The organic matrix containing the aesthetes was mounted on a microscope slide in lactophenol solution to form a temporary mount. These were then examined on a Reichert Zetopan photo-microscope.

For Scanning Electron Microscopy (SEM), when ventral or eave features were to be examined, pre-treatment of the valves with 5% KOH solution for 12 h was used to remove remains of tissues before dehydration and ultrasonic cleaning. Otherwise, valves were dehydrated through an acetone-alcohol series and cleaned ultrasonically for 10 sec prior to mounting on stubs. Treated valves were finally mounted on aluminium stubs and coated with gold palladium in a Polaron Equipment Ltd SEM Sputter coating unit E-5000. Specimens were examined at an accelerating voltage of 25kV on a Jeol JSM35 Scanning Electron Microscope.

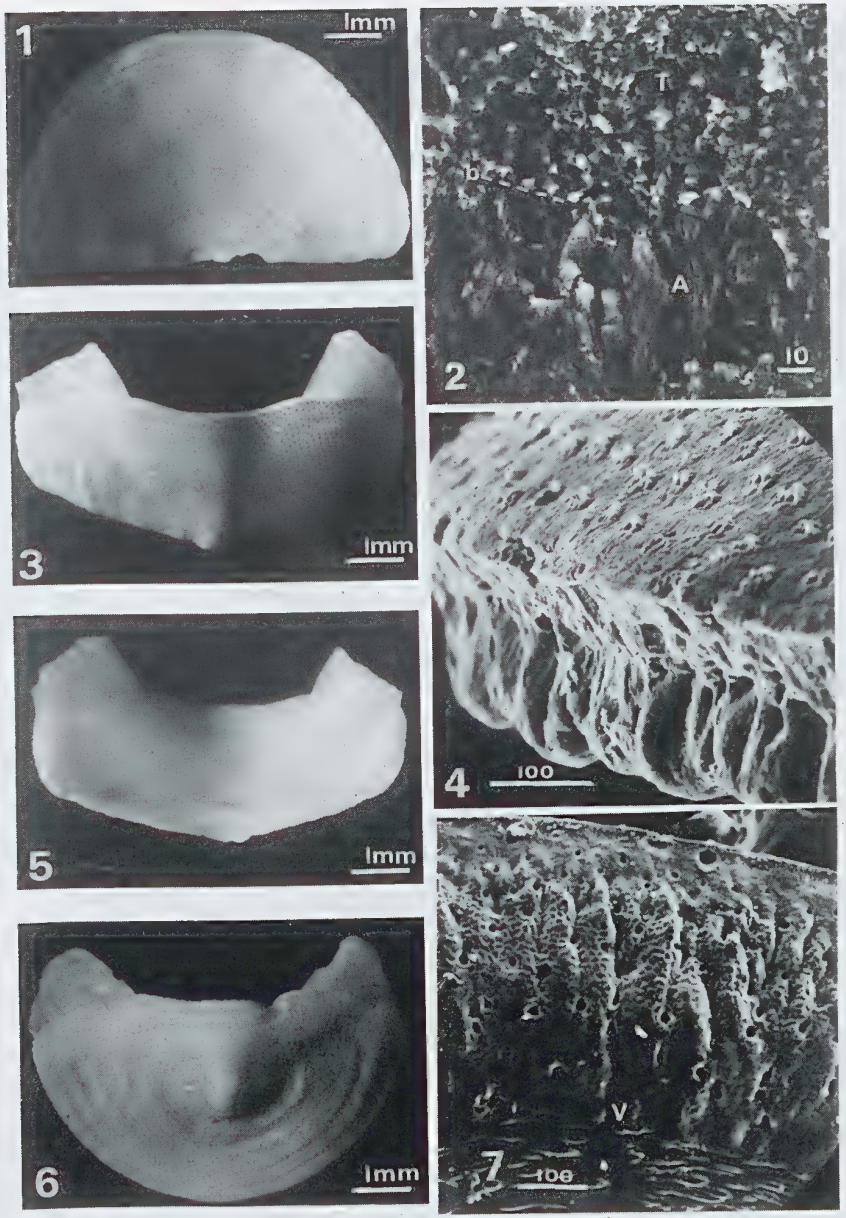
Samples of girdle for SEM examination were dissected out and prepared as described for examination above but without prior treatment with either EDTA or KOH solutions.

Valve Morphology

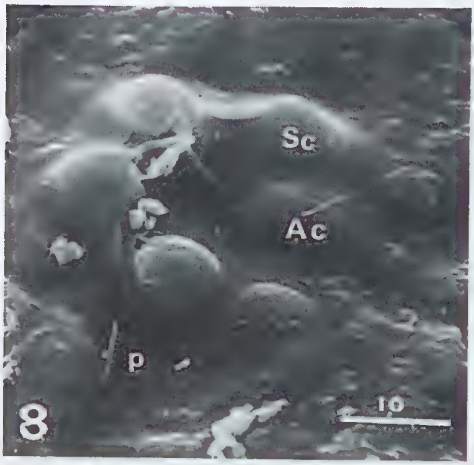
Xylochiton xylophagus typically has 8 valves which are rounded dorsally and of low elevation; one specimen was observed to have the left side of the 7th and 8th valves fused. The valves are strong, totally lacking in gross sculpture, and white in colour with darker spots visible in transmitted light (Figs 1,3), representing the positions of the aesthete groups described later. The tegmentum is seen in fractures (Fig. 2) to have a granular crystalline structure typical of this layer in other chitons, and through which the main aesthete channels run. The articulamentum (Fig. 2) has crossed lamellar crystalline structure, again typical of this layer in other species. There is a thin hypostracum visible also.

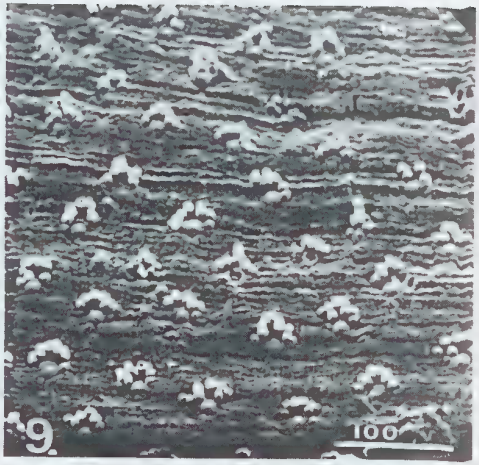
The anterior valve (Fig. 1) has a length:breadth ratio (L:B) of 0.5, and is smooth except for weak growth lines and evenly distributed granules which are the unpigmented aesthete groups. This valve bears small, unslit but distinctly corrugated insertion plates (Fig. 4) which are best developed on the fronto-lateral edges of the valve. The ventral surface bears occasional holes which are entry points for aesthete canals.

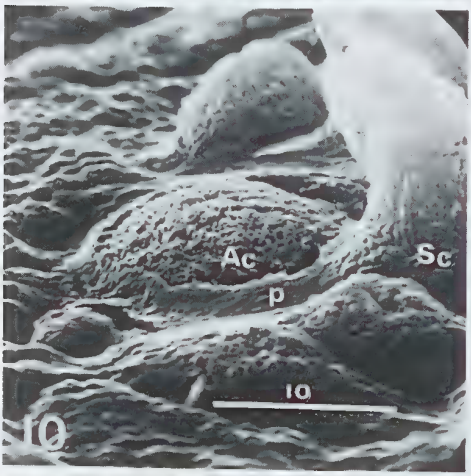
The intermediate valves (Fig. 3) are all weakly beaked with the posterior margin very slightly convex: the anterior margin is clearly concave with well developed sutural laminae which extend posteriorly into very small, weak insertion plates. The dorsal surface is undifferentiated, and only weak, concentric growth lines and the aesthete groups relieve the smooth valve surface. Valve 2 is approximately the same length as valve 1, but valves 3-7 are distinctly shorter in length. The ventral surface bears holes in the jugal area which are the openings for aesthete channels, and the tegmentum wraps under the posterior edge (Fig. 5) to form a distinct fold.

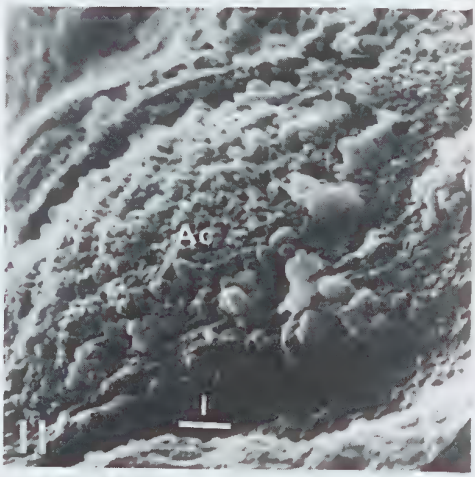


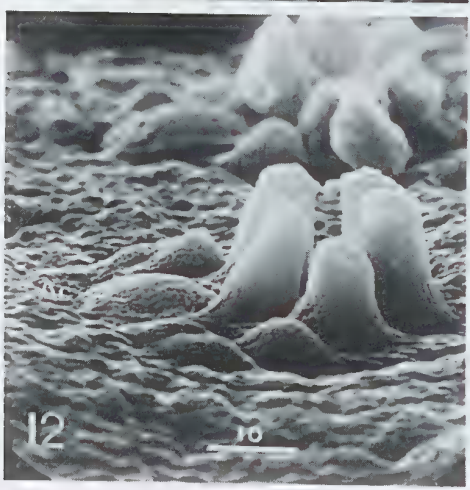
Figures 1-7. 1. Dorsal surface of the anterior valve. 2. SEM of dorso-ventrally fractured shell surface revealing the distinct boundary (b) between the granular tegmentum (T) and the plate-like appearance of the articulamentum (A). 3. Dorsal surface of intermediate valve 3. 4. SEM of the corrugated insertion plate found on the fronto-lateral areas of the anterior valve. 5. Ventral surface of intermediate valve 3 showing the distinct posterior fold. 6. Dorsal surface of the posterior valve showing growth lines. 7. SEM of posterior valve to show the thickened callus on the posterior ventral (V) surface. Scales in μm.

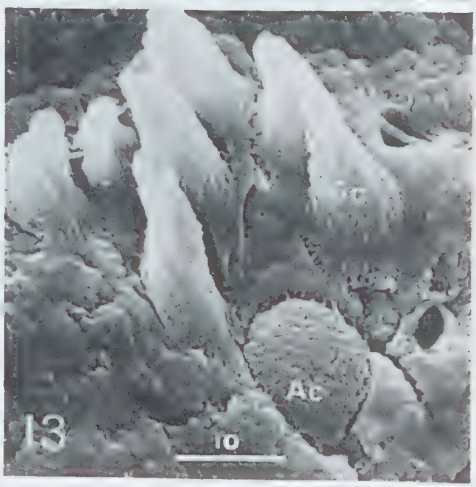

Sc
Ac
p
10
8


100
9


Ac
Sc
p
10
10


Ac
1
11


10
12


Ac
10
13

The posterior valve (Fig. 6) is similar in dimensions to the anterior valve, but bears strong sutural laminae and a distinct central mucro. Sculpturing is again lacking except for the concentric growth lines and the aesthete granules. The ventral surface is penetrated by a series of holes which are aesthete channel openings, and the articulamentum forms a thickened callus around the entire posterior margin (Fig. 7).

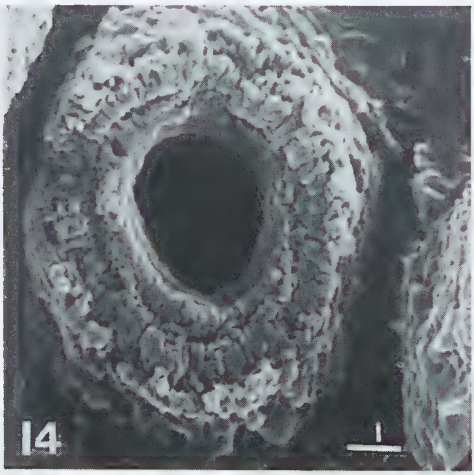
Microsculpture of the dorsal valve surface

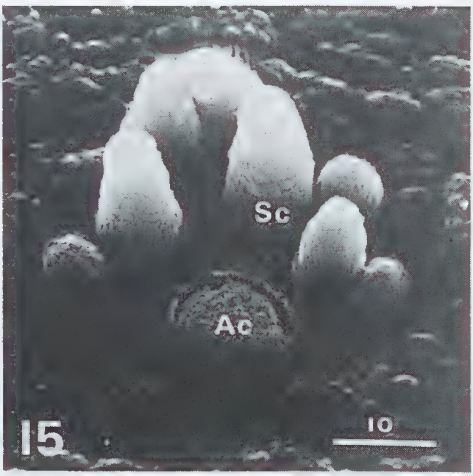
The outer surface of the valves in small animals has a distinct periostracal covering (Fig. 8), but in larger animals this is less distinct although visible on and around the aesthete structures (Fig. 10).

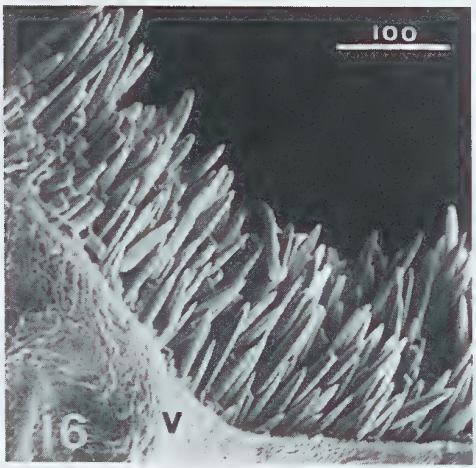
The dorsal valve surface bears no significant macrosculpturing other than that of broad growth lines visible on all valves. However, there is significant microsculpturing associated with both growth processes and the presence of aesthete groups. Figure 9 shows parallel microgrowth lines and the regularly arranged aesthete groups typical of this species, there being only minor and localised variations in this distribution pattern on all valves. The aesthetes have an average density of about 260/ mm^2 (n = 10; s.d. = ±12) in alternating rows forming a roughly diamond-shaped distribution pattern, with an average megalaesthete:micraesthete ratio of 1:7 (n = 100).

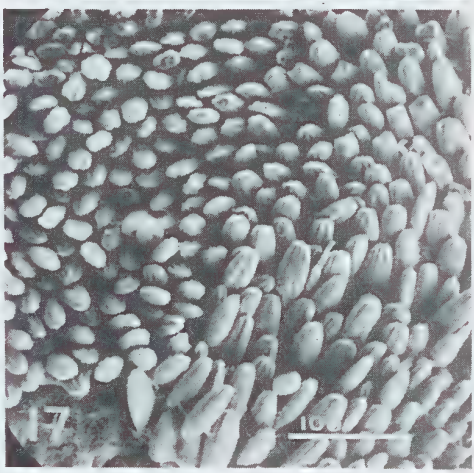
The principal aesthete components in *X. xylophagus* comprise typical elements: a series of mantle extensions entering from the lateral and anterior valve eaves form multiple branch channels (Baxter and Jones, 1981) which run horizontally through the tegmentum, giving off more or less vertical branches at regular intervals, each of which ends in a large megalaesthete chamber. A few jugal area type channels running vertically through both articulamentum and tegmentum are found on all valves. A single large apical cap extends from the megalaesthete chamber to the valve surface, together with a number of much smaller micraesthete branches which also terminate at the surface in micraesthete (subsidiary) caps. No comparison of the internal structure of megalaesthete or micraesthete components was possible due to the inappropriate fixation for TEM studies: light microscopy of wax sections did not indicate any significant differences from the generalised structures described by Boyle (1974). However, the surface structures and arrangements are of particular interest since they are apparently unique to these deep-water animals.

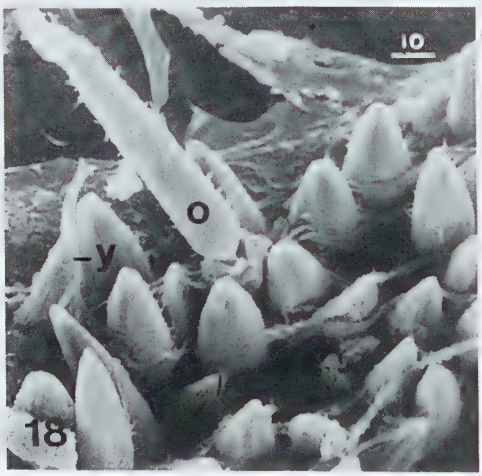
◀ **Figures 8-13.** **8.** SEM of valve surface of a very small animal with a thick and distinct periostracum intact over the valve surface. Note that the subsidiary caps (Sc) at this stage are not fully developed (Ac = apical cap). **9.** SEM of surface of anterior valve showing the microgrowth banding and the aesthete groups in a regular diamond-shaped arrangement. **10.** SEM of the apical cap of a fully developed aesthete group to show the perforated structure of the apical cap (Ac) and the residual parts of the periostracum (p) typically visible on and around the aesthete groups. **11.** Extreme enlargement of the surface of the apical cap (Ac) to show the apparent protrusion of blebs of material from the perforated apical cap surface. **12.** SEM of aesthete group viewed laterally to show the erect nature of the subsidiary caps in each aesthete group (Ac = apical cap). **13.** SEM showing the impact of short-term exposure of the valve to KOH solution. The result is the partial collapse of the subsidiary caps (Sc) and the exposure of the perforated nature of the apical cap (Ac). Scales in μm.


14
1


Sc
Ac
15
10


100
16
V


17
10


10
O
y
18

Each aesthete group comprises a slightly domed apical cap (Fig. 10), roughly circular in structure and averaging some 15μm in diameter: this cap is typical of those described for other species, being perforated in structure and with some evidence of secretory activity being visible in the form of blebs of material apparently being extruded (Fig. 11). The apical cap is surrounded by an almost semi-circular group of between 4 and 9 erect subsidiary caps (Figs 9, 12) which project up to 17μm above the valve surface and have a basal diameter of up to 10μm: these caps decrease in size towards the outer edge of each group and are always disposed towards the valve margin.

The subsidiary caps tend to shrink and collapse when treated with KOH solution (Fig. 13), and when seen in transverse section (Fig. 14) they reveal a clear annular structure with a distinct organic lining. Whether the caps are composed solely of organic matter is uncertain. The micraesthete cell body is absent due to the effects of processing for the SEM. The erect caps appear to have been produced by addition of material secreted outwards from the tip of each structure (Fig. 15), a conclusion substantiated by the observation that the subsidiary caps are very much lower in height on the growing edge than in older areas of the valves.

Shell growth

Shell growth in *X. xylophagus* is typical of that found in chitons from shallow water in that growth takes place mainly on anterior and lateral edges. There are both broad, uniformly spaced rings, possibly representing annual or spawning rings, as well as microgrowth bands seen under the SEM (Fig. 9). The latter form concentric bands following the growth patterns of each valve. The microgrowth bands are irregular in size and spacing, but clearly represent short-term variations in the biochemical processes used for laying down shell materials.

Girdle structure

The most striking feature of the girdle of *X. xylophagus* is that the ventral surface is devoid of any spiculation (Fig. 16), while the dorsal surface is densely spiculose with numerous, flattened boat-shaped spicules (Fig. 17). These spicules are 50-85μm long and 15-23μm wide, being more elongate towards the outer edge of the girdle. The spicules are orientated dorsally and are slightly concave on the inner surface, and the tips are weakly ridged (Fig. 17).

Sparse hair-like structures occur at random throughout the dorsal girdle (Fig. 18) and may be up to 100μm long and 12μm wide. The tips are markedly worn in all except the youngest examples where the tip appears to bifid (Fig. 18). The entire

◀ **Figures 14-18.** **14.** SEM section through a subsidiary cap showing the annular nature of the material forming the erect walls and the organic lining of the cavity, normally occupied by the micraesthete body. **15.** SEM of aesthete group to show the flowing nature of the subsidiary cap (Sc) growth layers (Ac = apical cap). **16.** SEM of the ventral surface of the girdle (V) showing its lack of spiculation and the elongate shape of the spicules at the periphery of the dorsal girdle surface. **17.** SEM showing the spiculation of the dorsal girdle surface. **18.** SEM detail of a portion of the dorsal girdle surface showing the spicules protruding from the cuticular covering of the girdle and showing the sparse, hair-like structures at two stages of development: the older one (O) can be clearly seen to have an articulated basal region while the younger stage (y) can be seen to have a bifid tip. Scales in μm.

structure projects from a short, 2-3μm wide stalk which appears to confer a flexibility to the structure which may have a sensory function.

DISCUSSION

The function of aesthetes has been the source of speculation and debate for many years. The original hypothesis relating to their functioning as light receptors (Mosely, 1885) is certainly valid for those species bearing specialised groups of aesthetes such as in *Callochiton septemvalvis* (Montagu, 1803) (Baxter and Jones, 1984 as *C. achatinus*), *Chiton* (*Diochiton*) *marmoratus Gmelin*, 1791 (Haas and Kriesten, 1978) and *Onithochiton neglectus* Rochebrune, 1881 (Boyle, 1969). Others have considered the structure of more generalised aesthetes such as in *Rhyssoplax olivacea* (Spengler, 1797) (Fischer and Renner, 1978 as *Chiton olivaceus*) and *Lepidopleurus cajetanus* (Poli, 1791) (Fischer, 1988) to contain photoreceptor cells also. An alternative hypothesis relating to a more generalised periostracum secreting function has been suggested by workers such as Baxter *et al.* (1987) for *Tonicella marmorea* (Fabricus, 1780), and attempts to account for the fact that where specialised aesthetes for light reception do occur, they only account for a relatively small proportion of the total aesthete complement, which probably, therefore, has a different function.

The subsidiary caps of *X. xylophagus* are very different from any yet described for other species of chitons, although initial observations by the authors suggest that erect subsidiary caps may also be found around the periphery of valves of another deep-water species, *Ferreiraella caribbensis* Sirenko, 1988 (family Abyssochitonidae). The functional significance of these erect subsidiary caps remains to be established, but their existence in at least 2 deep-water groups raises interesting questions with regard to both function and taxonomic status. More comparative SEM is needed on the deep-water species to determine how widespread such features may be.

The existence of distinct macro- and microgrowth lines is also of particular interest for a species apparently living and feeding on waterlogged wood in deep (>700m) water where relevant environmental cues would be difficult to identify, although seasonality in the deep sea is increasingly evident (Gage and Tyler, 1991). Microgrowth bands in shallow-water molluscan species (Richardson *et al.*, 1979) have always been related to tidal or day-length features, but their existence in deep-water chitons suggests that they may be an inherent biochemical feature of shell production, at least in these animals. The macro-growth bands are of undetermined frequency, and although they could be annual, they could equally be related to reproductive periods; their regularity would seem to rule out disturbance as a cause.

The stomach and intestine of all specimens of *X. xylophagus* examined were filled with very small wood fragments and a homogeneous material of undetermined origin. It is likely that wood is the primary material ingested, but whether or not the animals are capable of digesting the wood remains undetermined: the presence of apparently undamaged wood fibres in the posterior intestine suggests that the nutrition may be being obtained by the digestion of associated microflora/fauna rather than of the wood itself.

ACKNOWLEDGEMENTS

We would like to thank Mr B. Marshall (NMNZ) for initially bringing this material to our attention, and Mrs Margaret Gruber for assistance with preparation of photographs. Dr D. Currie is thanked for critical comments.

LITERATURE CITED

Baxter, J.M. & Jones, A.M. 1981. Valve structure and growth in the chiton *Lepidochitona cinereus* (Polyplacophora: Ischnochitonidae). J. Mar. Biol. Assoc. U.K. 61: 65-78.

Baxter, J.M. & Jones, A.M. 1984. The valve morphology of *Callochiton achatinus* (Mollusca: Polyplacophora: Ischnochitonidae). J. Zool. Lond. 202(4): 549-560.

Baxter, J.M., Jones, A.M. & Sturrock, M.G. 1987. The ultrastructure of aesthetes in *Tonicella marmorea* (Polyplacophora: Ischnochitonina) and a new functional hypothesis. J. Zool. Lond. 211: 589-604.

Boyle, P.R. 1969. Fine structure of the eyes of *Onithochiton neglectus* (Mollusca: Polyplacophora). Z. Zellforsch. 102: 313-332.

Boyle, P.R. 1974. The aesthetes of chitons. 2. Fine structure in *Lepidochitona cinereus* (L.). Cell Tissue Res. 153: 383-398.

Fischer, F.P. von. 1988. The ultrastructure of the aesthetes in *Lepidopleurus cajetanus* (Polyplacophora: Lepidopleurina). Amer. Malac. Bull. 6(1): 153-159.

Fischer, F.P. von & Renner, M. 1978. Die Feinstruktur der Ästheten von *Chiton olivaceus* (Mollusca, Polyplacophora). Helgolander Wiss. Meeresunters. 31: 425-443.

Gage J.D. & Tyler, P.A. 1991. Deep-Sea Biology: A natural history of organisms of the deep-sea floor. Cambridge University Press, Cambridge. 604pp.

Gowlett-Holmes, K.L. & Jones, A.M. 1992. Xylochitonidae fam. no., a new family of deep-water chitons, with descriptions of a new genus and species from New Zealand (Polyplacophora: Lepidopleurina). J. Malac. Soc. Aust. 13: 35-44.

Haas, W. & Kriesten, K. 1978. Die Ästheten mit intrapigmentarum Schalenauge von *Chiton marmoratus* L. (Mollusca, Placophora). Zoomorphologie 90: 253-268.

Mosely, H.N. 1885. On the presence of eyes in the shells of certain Chitonidae and on the structure of these organs. Q. Jl. Microsc. Sci. 25: 37-60.

Richardson, C.A., Crisp, D.J. & Runham, N.W. 1979. Tidally deposited growth bands in the shell of the common cockle, *Cerastoderma edule* (L.). Malacologia 18: 277-290.

Sirenko, B.I. 1988. A new genus of deep sea chitons *Ferreiraella* gen.n. (Lepidopleurida: Leptochitonidae) with a description of a new ultra-abyssal species. Zool. Zh. 67(12): 1776-1786. (In Russian).